

Paper Presentation

Superresolution by image scanning microscopy
using pixel reassignment

B10901151 林祐群
Instructor T. Tony Yang

Outline

- A. Overview
- B. Background
 - Fluorescent Microscopy
 - Confocal Microscopy
 - Image Scanning Microscopy
- C. Pixel Reassignment Graphical Interpretation
- D. Pixel Reassignment Mathematical Interpretation
- E. Stokes Shift
- F. Results
- G. Merits & Future Possibility
- H. References

Overview

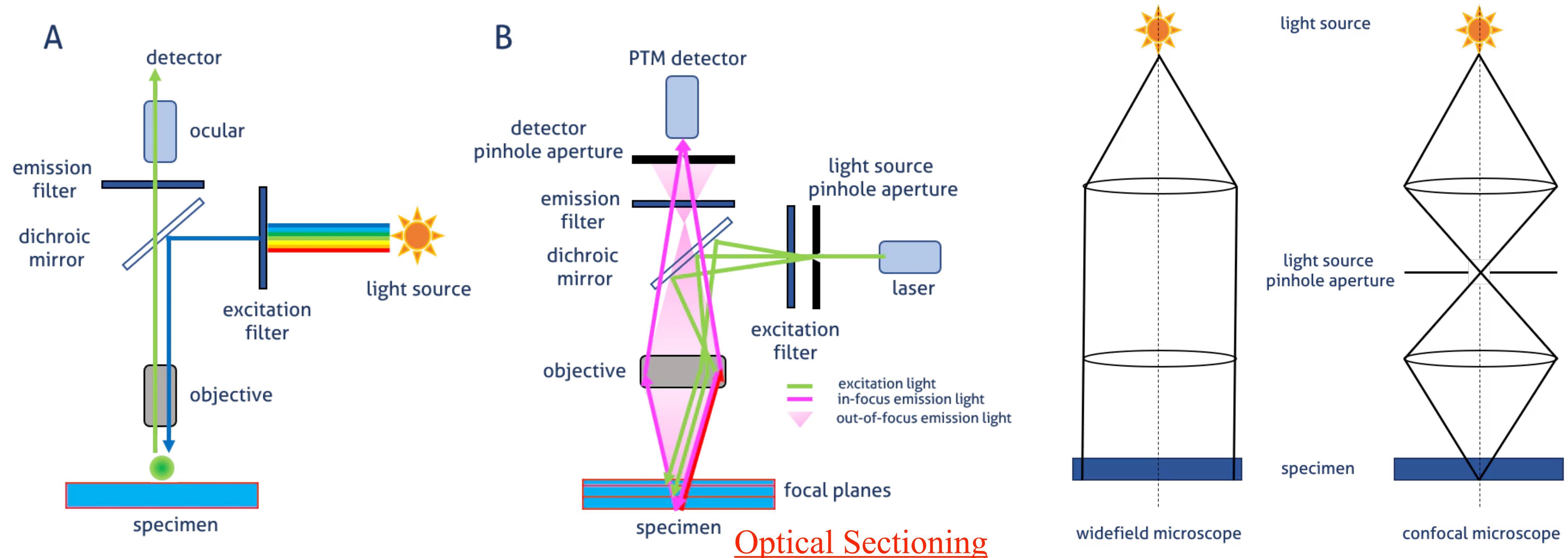
Superresolution by image scanning microscopy using pixel reassignment

Colin J. R. Sheppard, Shalin B. Mehta, and Rainer Heintzmann

Published July 30, 2013

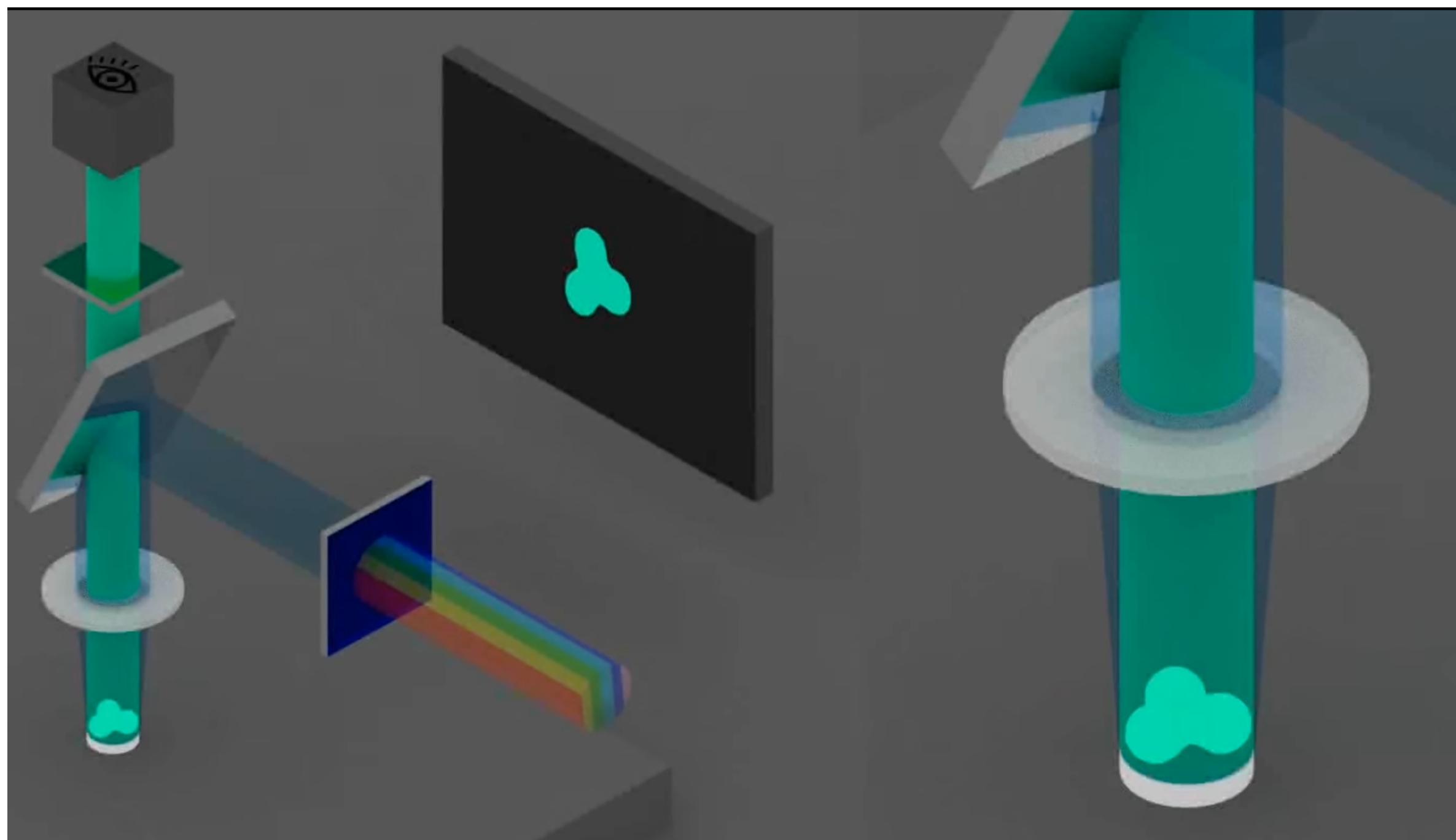
- Highlight the effect of **detector array size** on resolution and signal collection efficiency of image scanning microscopy based on **pixel reassignment** is studied.
- Describe the extension to fluorescent dyes exhibiting a **Stokes shift**.
 - A. The method can be employed if there is a Stokes shift in fluorescence emission wavelength.
 - B. With no Stokes shift, the width of the point spread function can be sharpened by a factor of 1.53, and its peak intensity increased by a factor of 1.84.

Fluorescent Microscopy & Confocal Microscopy



Reference[2]: <https://www.ptglab.com/news/blog/if-imaging-widefield-versus-confocal-microscopy/>

Fluorescent Microscopy & Confocal Microscopy



Fluorescent Microscope



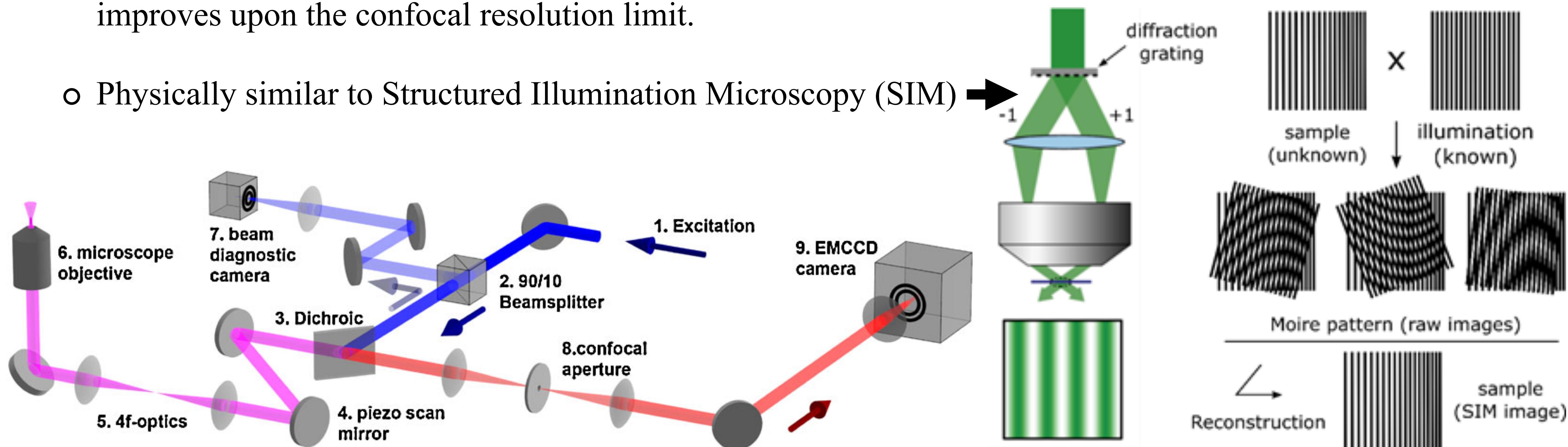
Confocal Microscope

Reference[3]: https://zh.wikipedia.org/wiki/File:Fluorescent_and_confocal_microscopes.ogv

Image Scanning Microscopy (ISM)

Claus B. Muller and Jorg Enderlein
Published 10 May 2010

- It greatly improves the detected signal level in confocal imaging, while at the same time slightly improves upon the confocal resolution limit.
- Physically similar to Structured Illumination Microscopy (SIM) →



Reference[4]: <https://physics.aps.org/featured-article-pdf/10.1103/PhysRevLett.104.198101>

Reference[5]: <https://andor.oxinst.com/learning/view/article/super-resolution-imaging-structured-illumination-microscopy>

Pixel Reassignment - I

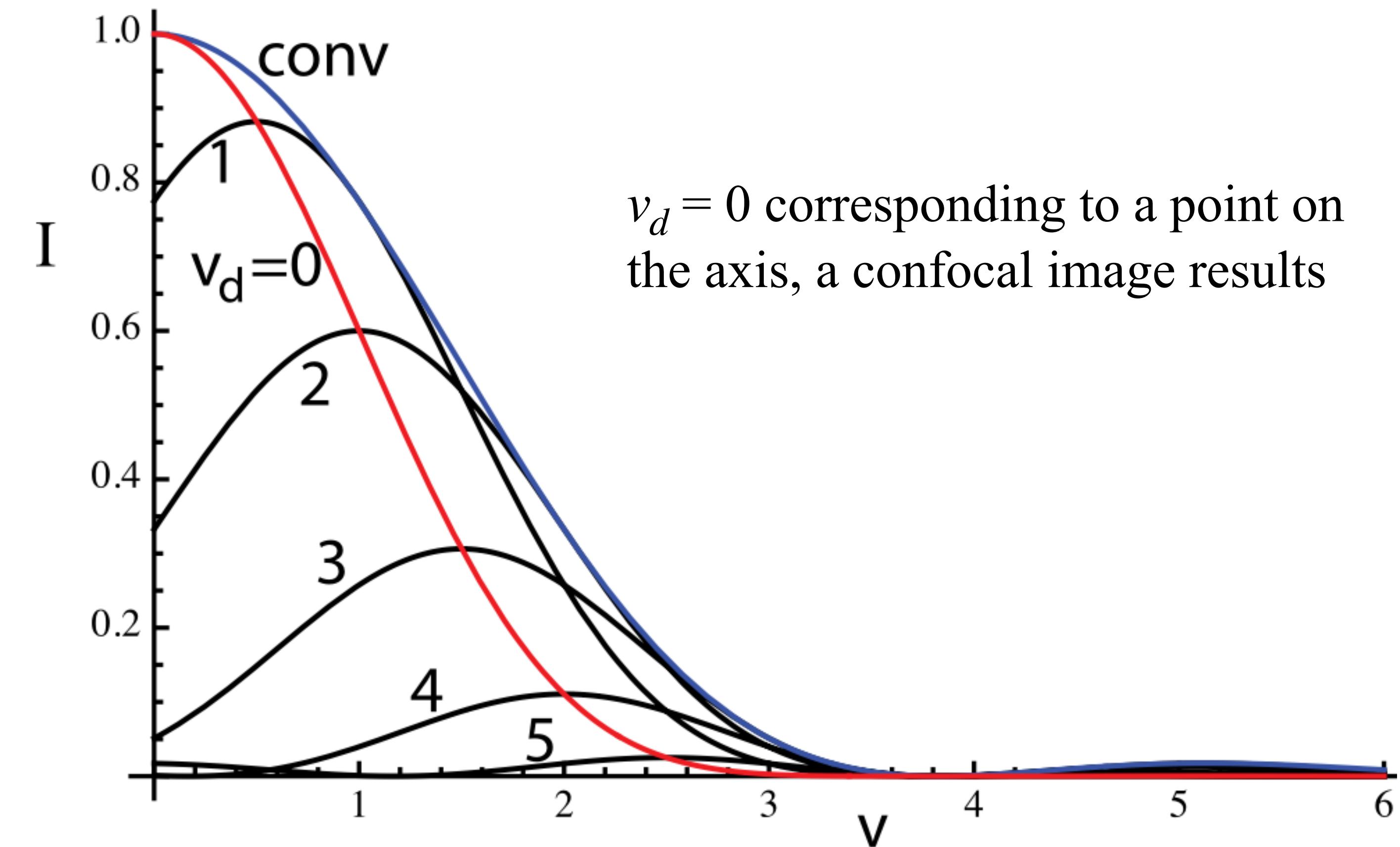
- Assume no Stokes shift here.
- Signal recorded from a **point object** at different points v_d in the detector plane as the sample is scanned.

Detector element position:

$$v_d = (2\pi/\lambda)x_d \sin \alpha_d$$

Normalized transverse coordinate of the scan position: $v = (2\pi/\lambda)nx \sin \alpha$

n: refractive index of immersion medium
α: semi-angular aperture of the microscope objective



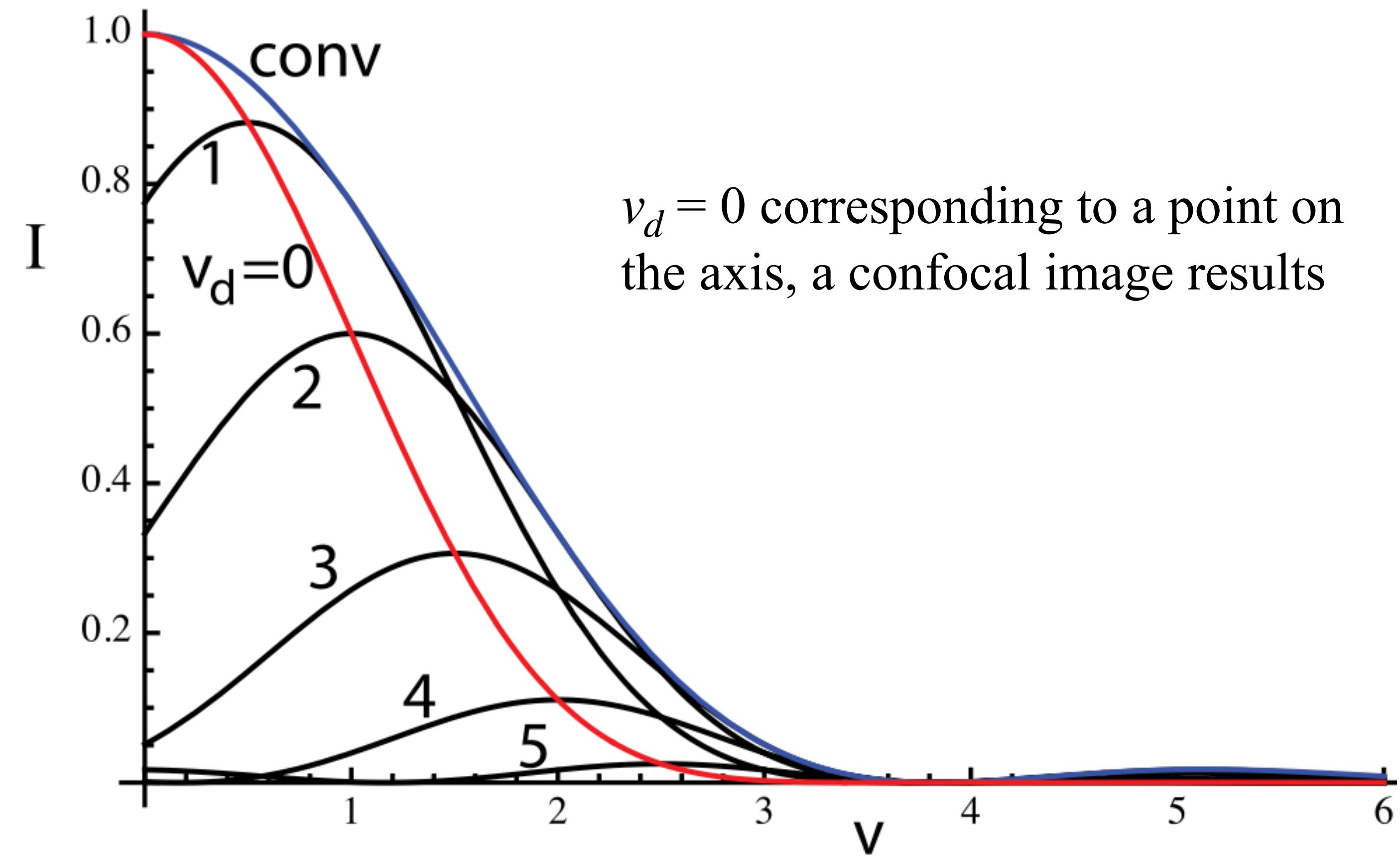
Pixel Reassignment - I

- Assume no Stokes shift here.
- Signal recorded from a **point object** at different points v_d in the detector plane as the sample is scanned.

As v_d increase:

1. Peak of image intensity decrease
2. Peak shifts sideways
3. Image is sharper
4. No longer circular symmetric

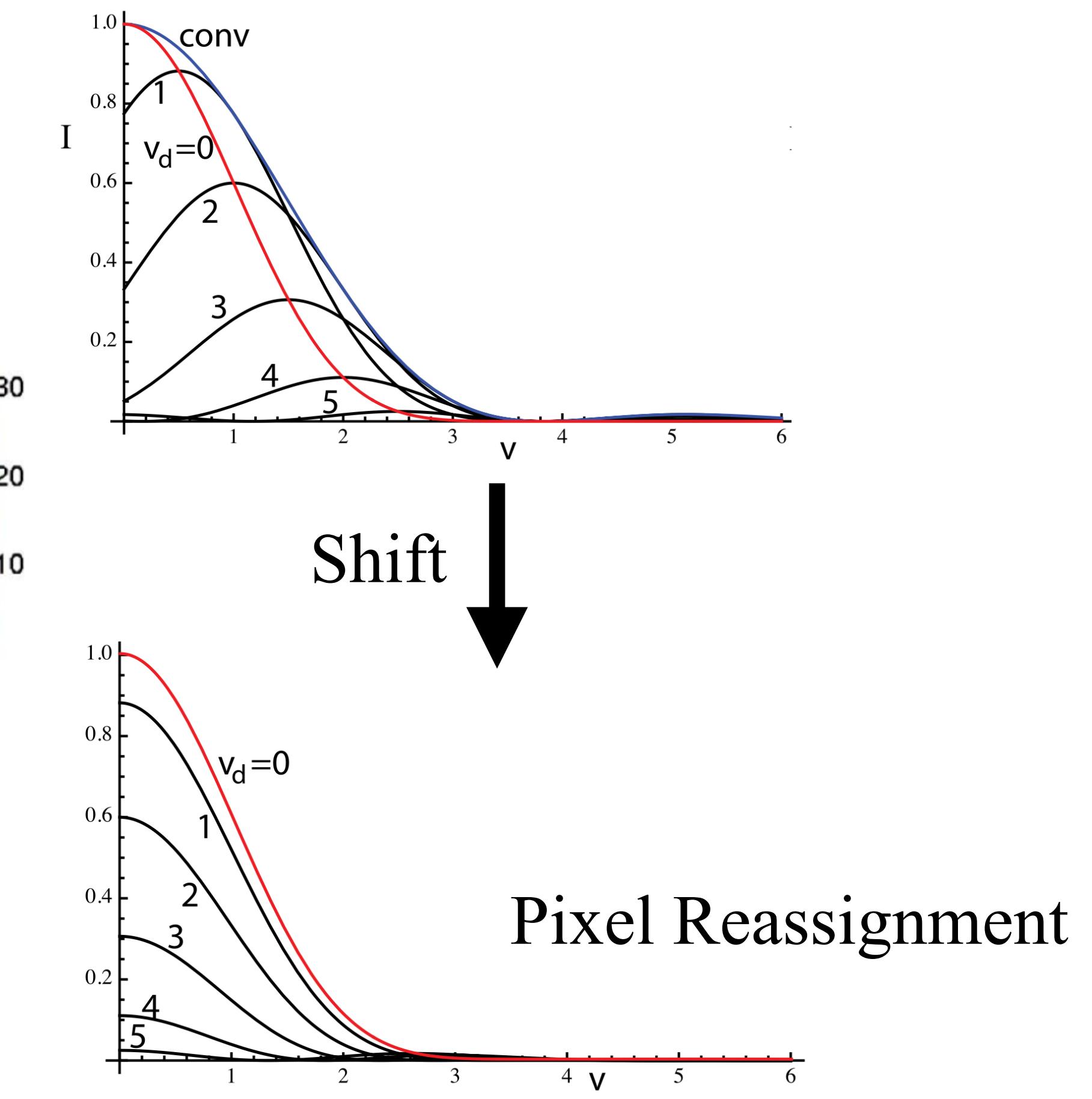
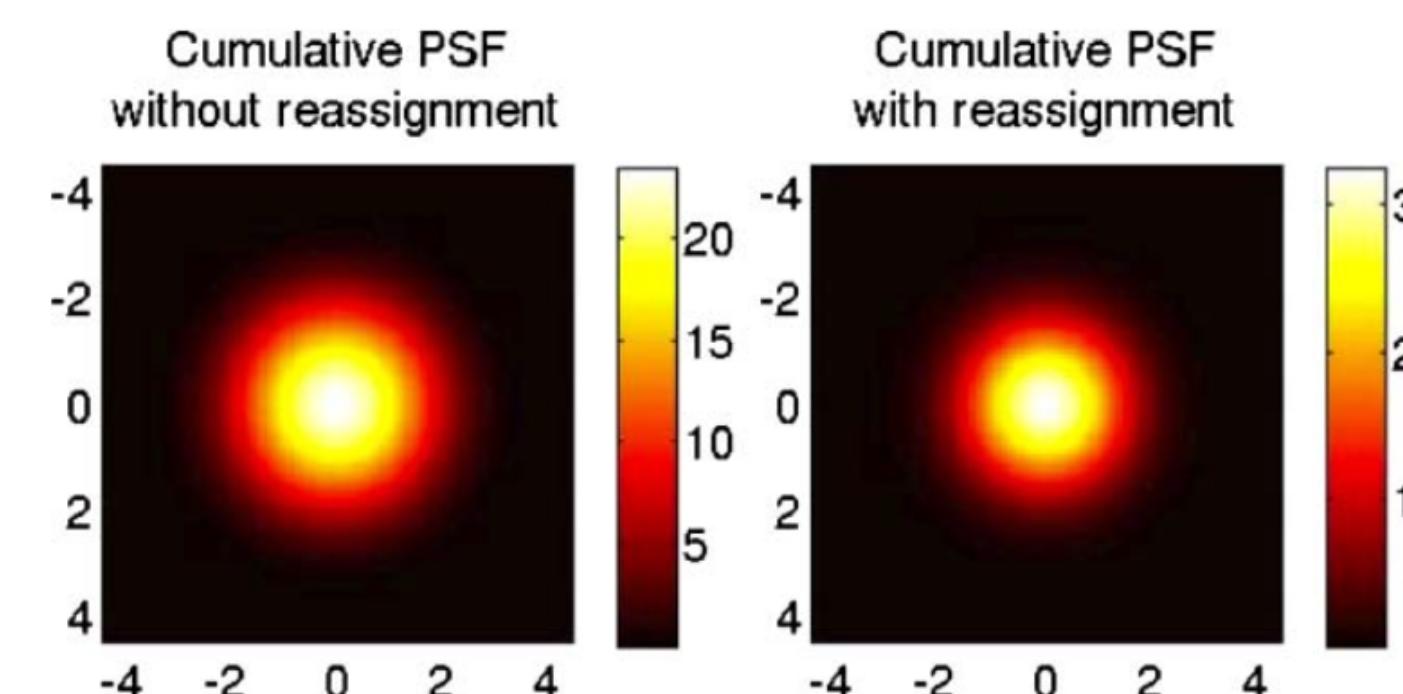
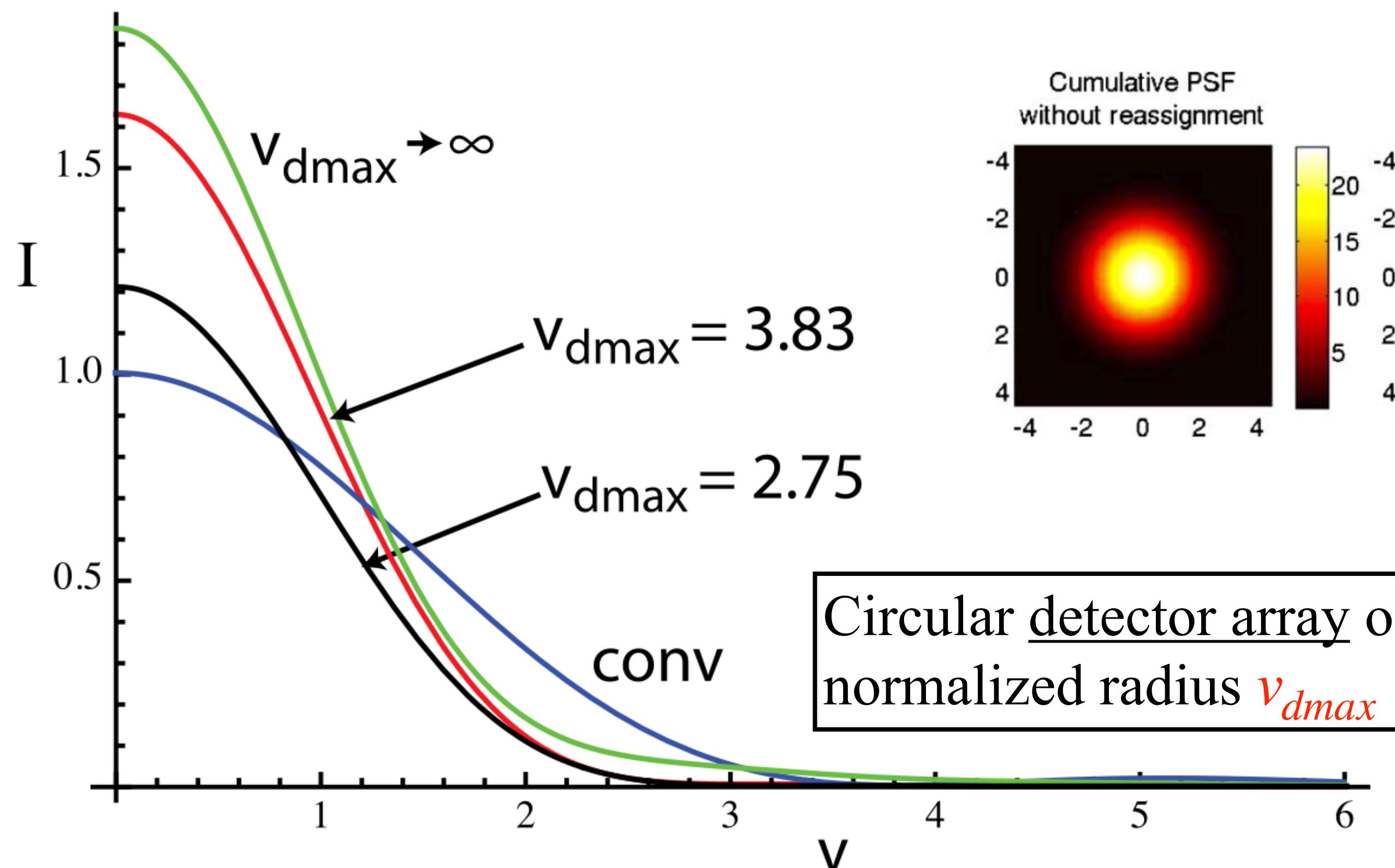
- Integration of these signals over the complete plane gives rise to nonconfocal image, equivalent to a conventional image.



$v_d = 0$ corresponding to a point on the axis, a confocal image results

Pixel Reassignment - II

- If the images from the off-axis detector points are shifted, they add up to give a sharper point spread function (PSF) i.e. **higher resolution**
- As v_{dmax} increases, the width of the PSF decreases monotonically



Pixel Reassignment - III

- To obtain this behavior, the values measured by a v_{dmax} must be summed to the image point $v = v_{dmax}/2$
=> Pixel Reassignment
- Detection efficiency:

$$\eta = \frac{\text{Output Signals}}{\text{Input Signals}}$$

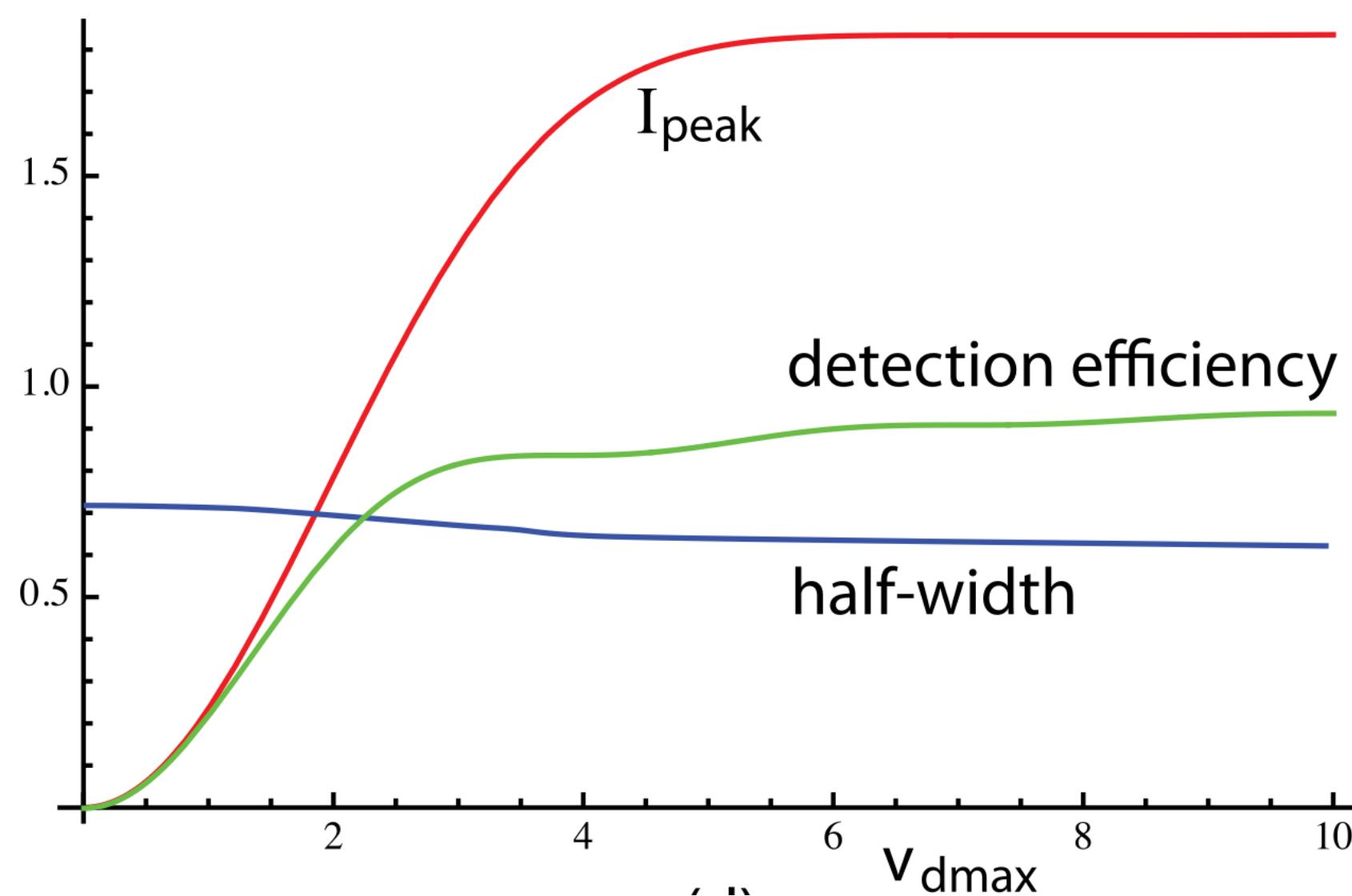


Table 1. Imaging Performance with Pixel Reassignment Compared with Conventional and Confocal Microscopes

	v_{dmax}	Normalized Half-width	Resolution Improvement Factor	Peak of PSF	η
Confocal	0	0.720	1.39	0	0
	2.75	0.689	1.45	1.21	0.79
	3.83	0.671	1.49	1.63	0.84
	∞	0.652	1.53	1.84	1
Conventional		1	1	1	1

The improvement in resolution, defined in terms of the **half-width of the PSF**

Mathematical Perspective

- $I(\mathbf{x}_s, \mathbf{x}_d) = \iint H_1(\mathbf{x}') F(\mathbf{x}' - \mathbf{x}_s) H_2(\mathbf{x}' - \mathbf{x}_d) d^2\mathbf{x}'$

(Substitute $\mathbf{x}_s = -\mathbf{x}_1$, $\mathbf{x}_d = \mathbf{x}_2 - \mathbf{x}_1$, $\mathbf{x}' = \mathbf{x} - \mathbf{x}_1$) corresponding to **scanning the illumination beam instead of the specimen**)

- Can be written in symmetrical form for the four-dimensional signal:

$$I(\mathbf{x}_1, \mathbf{x}_2) = \iint H_1(\mathbf{x} - \mathbf{x}_1) H_2(\mathbf{x} - \mathbf{x}_2) F(\mathbf{x}) d^2\mathbf{x}$$

Notation:

x_s : scan position

x_d : pixel position on detector

$F(\cdot)$: Fluorescent Distribution on sample

H_1 : illumination PSF

H_2 : detection PSF

- The signal from measurement \mathbf{x}_d reassigned to the point $\mathbf{x}_r = (1 - a)\mathbf{x}_1 + a\mathbf{x}_2$ and summed with pinhole weighting factor $S(\mathbf{x}_d)$ (1 inside a specific radius, 0 otherwise):

$$I(\mathbf{x}_1, \mathbf{x}_2) = \iiint H_1[\mathbf{x} - \mathbf{x}_r - a\mathbf{x}_d] H_2[\mathbf{x} - \mathbf{x}_r + (1 - a)\mathbf{x}_d] \times F(\mathbf{x}) S(\mathbf{x}_d) d^2\mathbf{x} d^2\mathbf{x}_d \quad \forall a \text{ with } 0 \leq a \leq 1$$

High-Level Perspective:

x_1 : excitation focus position

x_2 : conjugate position on detector to object space

Mathematical Perspective

- $I(\mathbf{x}_1, \mathbf{x}_2) = \iiint H_1[\mathbf{x} - \mathbf{x}_r - a\mathbf{x}_d]H_2[\mathbf{x} - \mathbf{x}_r + (1 - a)\mathbf{x}_d] \times F(\mathbf{x})S(\mathbf{x}_d) d^2\mathbf{x} d^2\mathbf{x}_d \quad \forall a \text{ with } 0 \leq a \leq 1$

$a = 0$	The system becomes a scanning (nonconfocal) microscope, equivalent to integration without reassignment
$a = 1/2$	Pixel reassignment as described above
$a = 1$	The system reduces to a conventional microscope

- If $S(\mathbf{x}_d) = 1$, **using a large detector**, the integral in \mathbf{x}_d gives the effective PSF as the 2D **convolution** of two scaled, 3D PSFs (illumination and detection PSFs). Thus, $C_{eff}(\mathbf{m})$ is the **product** of two scaled, illumination and detection, optical transfer functions (OTFs) in x, y , convolved **in the axial direction**.

$$C_{eff}(\mathbf{m}) = C_1[(1 - a)\mathbf{m}]C_2[a\mathbf{m}], \text{ with } \textit{in-plane spatial frequency } \mathbf{m}$$

C_1 : Excitation Transfer Function
 C_2 : Emission Transfer Function

Mathematical Perspective

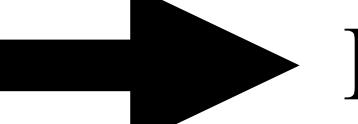
x, y directions: Product of two scaled OTF

z direction: 1D Convolution

- $C_{eff}(\mathbf{m}) = C_1[(1 - a)\mathbf{m}]C_2[a\mathbf{m}]$, with *in-plane spatial frequency* \mathbf{m}

C_1 : Excitation Transfer Function

C_2 : Emission Transfer Function

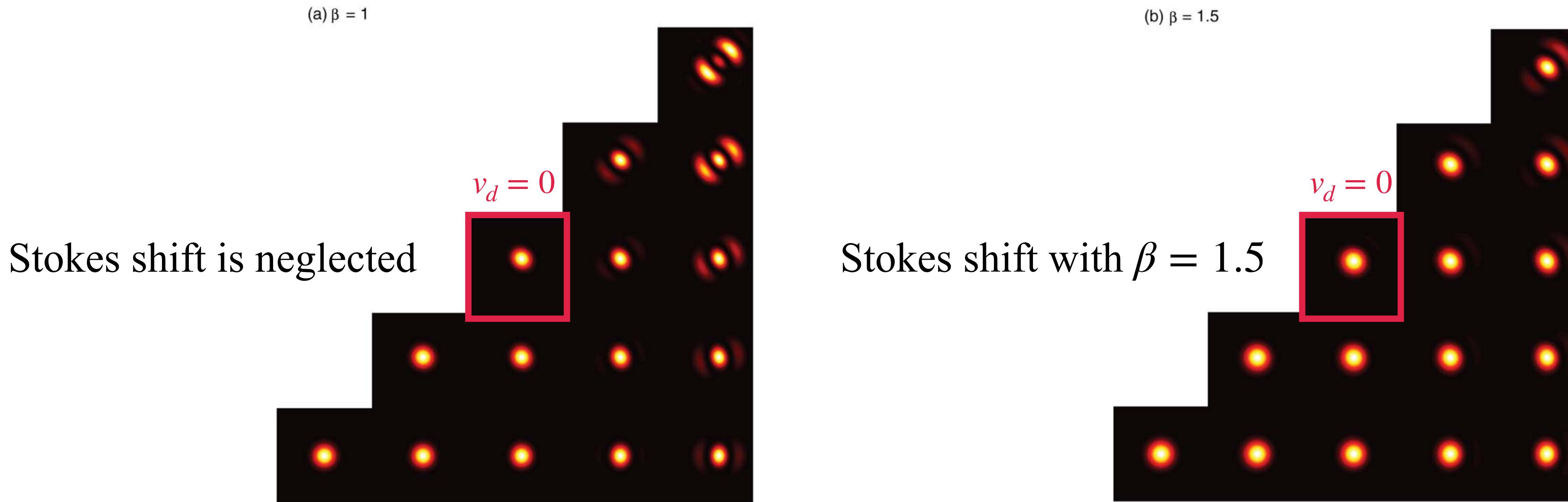
Pro: The cutoff spatial frequency is doubled  Doubled the resolution in transverse directions

Con: As $C_1(0) = C_2(0) = 1$, **independent of the presence of defocus**, $C_{eff}(0) = 1$, so that there is **no optical sectioning** in axial direction when pixel reassignment is used with a large detector array.

- Optical sectioning can be regained by limiting the size of the detector array. (e.g. $v_{dmax} = 2.747$)
- $v_{dmax} = 3.83$ corresponds to a detector array equal in size to the first dark ring of the Airy disk.
- Suitable for imaging of the projection of a thin sample.

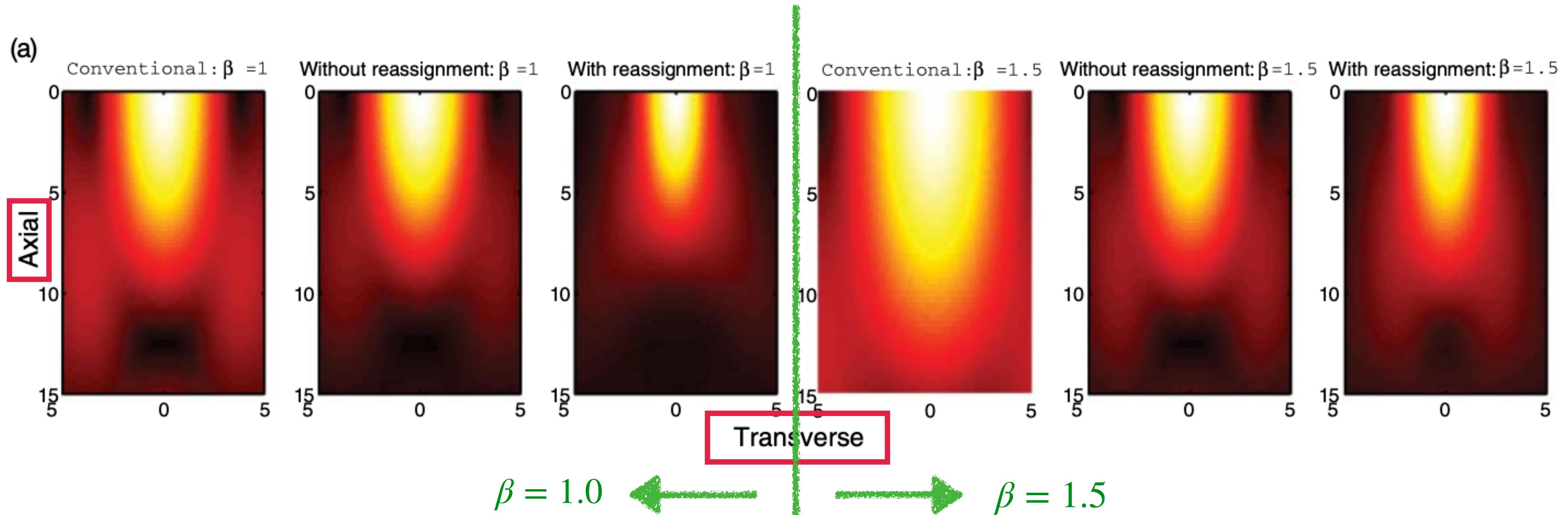
Stokes Shift

- If there is Stokes shift, we choose $a = \frac{1}{1 + \beta}$, where $\beta = \frac{\lambda_{emission}}{\lambda_{excitation}}$
- Thus, we find that $C_{eff}(\mathbf{m}) = C_1\left(\frac{\beta\mathbf{m}}{1 + \beta}\right) + C_2\left(\frac{\mathbf{m}}{1 + \beta}\right) \Rightarrow$ the cutoff frequency improves by a factor of $(1 + \beta)$ compared with a conventional microscopy.



Results - PSF

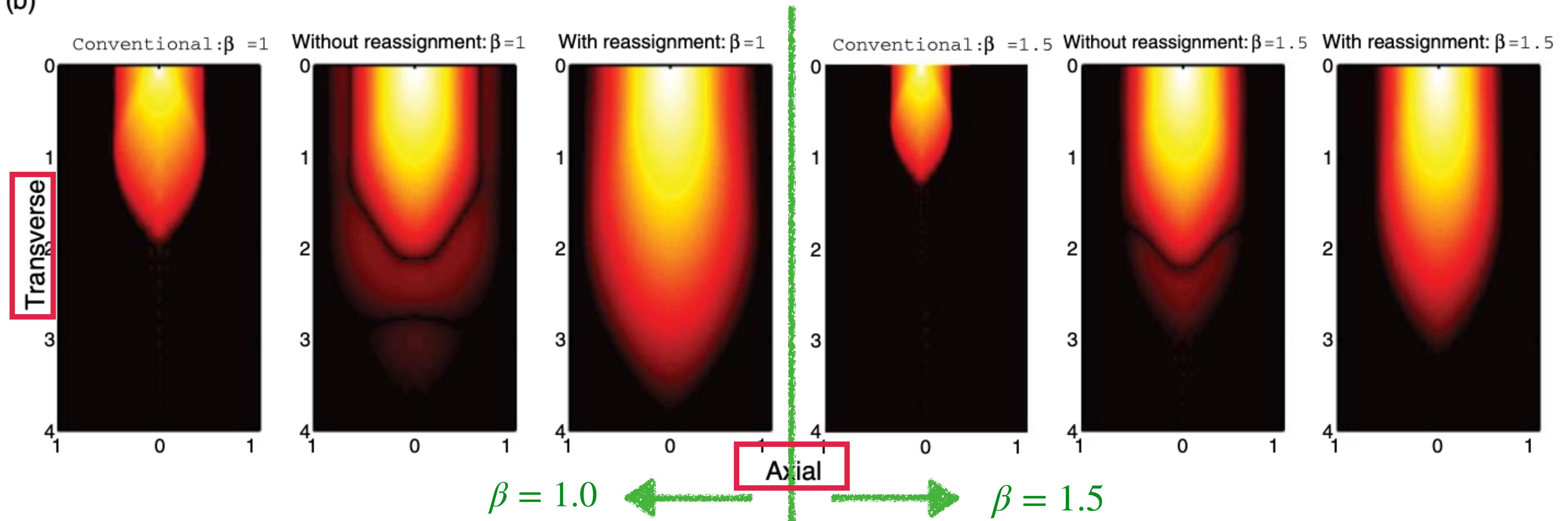
- Reassignment of signal by av_d can be **performed without interpolation** by using scan pixels of size $\frac{a}{N}$ (N integers), the size of the detector pixel. \Rightarrow better efficiency
- Reassignment provide better resolution and SNR.



Results - OTF

- Reassignment of signal by av_d can be **performed without interpolation** by using scan pixels of size $\frac{a}{N}$ (N integers), the size of the detector pixel. \Rightarrow better efficiency
- Reassignment provide better resolution and SNR.

(b)



Merits & Future Possibility

Pixel Reassignment:

- Has higher signal collection.
- Can collect better high frequency information than confocal microscopy
- Can achieve even better resolution in the case with stokes shift.
- Can combine with other post-processing or algorithms with flexibility.
- It is possible to implement the reassignment concept in an all-optical way.
 - Requiring the recording of only a single frame
 - Improved speed and reduced read noise

Future Possibility:

- Combine with modern techniques to achieve better performance.
- Achieve real-time super resolution imaging.
- Consider multi-photon excitation instead of the proposed single photon.

References

[1] Superresolution by image scanning microscopy using pixel reassignment

<https://opg.optica.org/ol/abstract.cfm?uri=ol-38-15-2889>

[2] IF imaging: Widefield versus confocal microscopy. An overview of microscopy techniques and advice on when to use each method.

<https://www.ptglab.com/news/blog/if-imaging-widefield-versus-confocal-microscopy/>

[3] Fluorescent Microscopy and Confocal Microscopy

https://zh.wikipedia.org/wiki/File:Fluorescent_and_confocal_microscopes.ogv

[4] Image Scanning Microscopy

<https://physics.aps.org/featured-article-pdf/10.1103/PhysRevLett.104.198101>

[5] Structured Illumination Microscopy

<https://andor.oxinst.com/learning/view/article/super-resolution-imaging-structured-illumination-microscopy>